OPUS® Pre-packed Chromatography Columns

Regulatory Support File

Supplement A: Extractables and Leachables

RSF-Supplement A

REPLIGEN

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Contents

List of tables

Abbreviations

amu	atomic mass unit
BPOG	BioPhorum Operation Group
BPSA	Bio-Process Systems Alliance
C	Celsius
CFR	Code of Federal Regulations
cm	centimeter
E&L	extractables and leachables
EMA	European Medicines Agency
EtOH	ethanol
g	gram
GC	gas chromatography
ICP	Inductively Coupled Plasma
LC	liquid chromatography
M	Molar
mL	milliliter
MS	mass spectrometry
N	Normal
N/A	not applicable
NaCl	sodium chloride
NaOH	sodium hydroxide
NMR	nuclear magnetic resonance
OPUS	Open Platform User Specified
PDA	photodiode array
PDA	Parental Drug Association
PEG	polyethylene glycol
PET	polyethylene terephthalate
PP	polypropylene
PQRI	Product Quality Research Institute
TSE	transmissible spongiform encephalopathy
UPLC	ultra-performance liquid chromatography
USP	United States Pharmacopeia
µg	microgram

1. Introduction to Extractables and Leachables

Preferred plastics used in biopharmaceutical manufacturing have minimal defined extractables and have been determined to be non-toxic at equivalent therapeutic doses. Many base standards used for guidance have been set by regulatory publications including USP, CFR 21, and European Medicines Agency (EMA). These basic standards have been elaborated on by industry organizations like the Bio-Process Systems Alliance (BPSA) and Parenteral Drug Association (PDA) as well as product manufacturers through the publication of best practices of testing and assessment of data. In addition, end user therapeutic manufacturers have become more demanding in their analytical requirements, assessment of data, determination of risk, and minimum threshold for meeting internal standards.

There is a consensus that it is the responsibility of the product technology vendor to provide an extractable data package. Thus, Repligen has conducted an extractables study on the plastics used in the product contact components of OPUS® Columns. In accordance with industry standards, Repligen uses the definitions for extractables and leachables as stated in the 2007 BPSA document, *"Recommendations for Extractables and Leachables Testing: Part 1."*

Extractables: Chemical compounds that migrate from any product contact material when exposed to an appropriate solvent under exaggerated conditions of time and temperature.

Leachables: Chemical compounds, typically a subset of extractables, that migrate into a drug formulation from any product contact material as a result of direct contact under normal process conditions.

In recent years there have been efforts to standardize the testing procedures for extractables, and wherever possible we intend to comply with the standardization efforts. In addition, in compliance with CFR 21, Part 211.65, OPUS Columns are designed such that all product contact materials are not reactive, additive, or absorptive as to not alter the safety, identity, strength, quality, or purity of the drug product beyond established requirements.

Therefore, using the BPSA guidance as a backdrop, Repligen has designed an extractables program to produce a robust data package by conducting extractables testing using three solvents over exaggerated time and temperature conditions. Solvents were chosen for their common usage in chromatography columns. Leachables, however, are considered to be process specific and the responsibility of the end user to define within specific process parameters.

1.1 OPUS Column Strategy: Extractables

Based on the above discussion, Repligen has applied the following philosophy in its approach to developing a meaningful extractables and leachables package for OPUS Columns:

1. All plastics used will be certified to meet four criteria:
    A. USP <88> Biological Reactivity Tests, In Vivo (USP Class VI)
    B. 21 CFR Part 177 Indirect food Additives: Polymers
        i. Section 177.1520 Olefin polymers (polypropylene)
        ii. Section 177.2600 Rubber articles intended for repeated use (silicone)
    C. USP general chapters <661>: Polypropylene (PP) Containers

Certified animal-free or compliant with EMA 410/01
Extractables from the OPUS 2.5 – 80R Column platform were determined according to an approved written experimental rationale for exaggerated time and temperature conditions and tested against an approved protocol.

1.2 OPUS Column Strategy: Leachables

The extractables information in this regulatory support file is designed to be the foundation of a leachable substance's therapeutic safety assessment. The data presented represent an extractables profile of what might be extracted from an OPUS Column under extreme exaggerated conditions (prolonged exposure at elevated temperature).

Role of the end user
Each process is different, and therefore must be evaluated for impact of leachables. In many cases, the data provided by an extractables test can be used to create a risk-based assessment to support a leachables program.

Role of Repligen
Repligen will neither conduct nor present data for leachables in this Regulatory Support File for any process-specific solution beyond what is determined in the extractables testing. Repligen will provide, on request, suitable component test materials to support application- and process-specific leachables testing.

1.2.1 Considerations for Leachables Testing

Purpose
To assess the safety risk posed to patients through exposure to low levels of chemical entities extracted from plastics in the manufacturing process.

Role of the model solvent extraction test
The model extraction vehicles in a standard extractables test will likely show all the materials that could be extracted from a plastic product. Model stream extraction vehicles are chosen for their ability to extract compounds from plastics, as well as their relevance to the test application (i.e., chromatography). The results of extractable tests can be compiled and analyzed to create a risk-based approach for conducting (or not conducting) a leachables study.

Chromatography: an assessment of application impact
For chromatography, the method from start to finish should be considered; however, a risk analysis should focus on the elution fluid, which, in most cases, will be an aqueous solution. Although a pre-packed chromatography column will see solvents such as 0.5 N sodium hydroxide, phosphoric acid, high and low pH additives, and high salt conditions, in general, none of these compounds or additives are meant to pass into the final product pool. In addition, impact to the final product pool is limited due to multiple chromatography or filtration steps that help to eliminate extractables throughout a typical downstream process. Therefore, to assess the impact of additional solvents, extractables and leachables studies can be carried out with the proposed purification methods in mind. This will enable a thorough understanding of the impact of the chromatography steps as it pertains to leachables in the final product pool.

1.3 USP <88> Biological Reactivity Tests, *In Vivo*

Six plastic classes are defined in Table 1. These classifications are based on responses to a series of in vivo tests for which extracts, materials, and routes of administration are specified. These tests are directly related to the intended end-use of the plastic articles.

Table 1. Summary of USP <88> plastics classes

Plastic classes						Tests to be conducted		
I	II	III	IV	V	VI	Extract	Species	Procedure
X	X	X	X	X	X	USP 0.9% NaCl	Mouse	Systemic-Intravenous
X	X	X	X	X	X		Rabbit	Intracutaneous irritation
	X	X	X	X	X	1:20 EtOH/NaCl	Mouse	Systemic-Intravenous
	X	X	X	X	X		Rabbit	Intracutaneous irritation
		X		X	X	PEG 400	Mouse	Systemic-Intravenous
				X	X		Rabbit	Intracutaneous irritation
		X	X	X	X	Cottonseed oil	Mouse	Systemic-Intravenous
			X	X	X		Rabbit	Intracutaneous irritation
			X		X	Muscle implant	Rabbit	Intramuscular (7 day, no histopathology)

Acute systemic toxicity test
Purpose: In vivo systemic tests evaluate the impairment or activation of a system rather than the impairment of individual cells or organs. In acute systemic toxicity tests, the test material (extract) is tested for systemic toxic effects after a single, acute exposure. This test is designed to evaluate systemic responses to the extracts of materials following injection into mice.

Irritation test: Intracutaneous irritation test
Purpose: The irritation tests are in vivo screening tests to evaluate the potential of test materials or their extracts to cause irritation on the exposed part of the body. This test is designed to evaluate local responses to the extracts of materials following intracutaneous injection into rabbits.

Implantation Test: Intramuscular
Purpose: Implant studies evaluate the local pathological effects on living tissue at both the gross and microscopic level of a test article surgically implanted into an appropriate implant site. The implantation test is designed for the evaluation of plastic materials and other polymeric materials in direct contact with living tissue.

1.4 CFR 21 Part 177 Indirect Food Additives: Polymers

Subpart B - Substances for Use as Basic Components of Single and Repeated Use Food Contact Surfaces.

- Section 177.1520 – Olefin polymers (applied to polypropylene) states that may be safely used as articles or components of articles intended for use in contact with food, subject to the provisions of this section.

Subpart C - Substances for Use Only as Components of Articles Intended for Repeated Use.

- Section 177.2600 – Rubber articles intended for repeated use (applied to silicone) may be safely used in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food, subject to the provisions of this section.

1.5 USP General Chapter <661>: Polypropylene Containers

The standards and test provided in this section characterize polypropylene containers produced from homopolymers or co-polymers that are suitable for packaging dry solid or liquid oral dosage forms.

1.6 Animal Free and EMA 410/01

Note: *This is for guidance on minimizing the risk of animal transmissible spongiform encephalopathy (TSE) agents via human and veterinary medicinal products.*

Scientific principles for minimizing risk (EMA 410/01): Use of materials from non TSE-relevant animal species or non-animal origin is preferred. If materials from TSE-relevant animal species must be used, consideration should be given to all the necessary measures to minimize the risk of transmission of TSE.

2. Extractable Test Protocol for OPUS Columns

The following report was generated by Michael Roberto, Ph.D. (Material Needs Consulting, Medical Device and Packaging Services).

Background
Repligen has performed polymer deformulation and extractables testing on the product contact components of their OPUS Columns. This data has been consolidated into a format that will be suitable for use in the OPUS product literature.

Materials
The materials used to fabricate single use processing equipment for biopharmaceutical manufacturing are often polymers, such as plastic or elastomers (rubber), as opposed to the traditional metal or glass. Polymers offer more versatility, since they are light-weight, flexible and much more durable than their traditional counterparts. Plastic and rubber are also disposable, so issues associated with cleaning and its validation are often avoidable. Whenever there is contact between a material of construction for a manufacturing component and therapeutic, there is always the possibility for chemicals from the material to migrate or leach into the drug. All materials can produce leachables.

Leachables are the chemicals that migrate from single-use processing equipment into the various components of the drug product during manufacturing. Extractables are chemicals entities, organic and inorganic, that can be extracted from single-use processing equipment using common laboratory solvents in controlled experiments. They represent the worst-case scenario and are used as a tool to predict the types of leachables that can be encountered during pharmaceutical production. So, extractables are the potentials and leachables are the actuals.

An effective way to minimize and control leachables is to use well characterized materials to fabricate the manufacturing equipment. The OPUS Column product contact materials are mainly constructed from polypropylene (PP) homopolymers and platinum cured silicone. PP homopolymer is much less prone to leachables than other types of polyolefins due to its mechanical and physical properties (such as melting point, glass transition temperature, molecular weight distribution, and percent crystallinity.) All of these important polymer characteristics have been measured and benchmarked for each PP product contact component and can be used as a quality control measure to ensure that all incoming raw materials have similar properties. This can help to ensure consistent, batch-to-batch leachables profiles for the OPUS product contact components. The additives package has also been determined for each polymer in an effort to predict and control leachables. The platinum cured silicone components are exceptionally durable materials that are often used in medical applications. These silicone elastomers are amorphous polymers that usually do not require formulation with additives such as phthalates, antioxidants, or heat and light stabilizers. This less complex formulation greatly reduces the risk of leachables. The silicone polymers have also been fortified with silica fillers to provide the essential mechanical properties required for the OPUS chromatography applications. As in the case of the PP components, the mechanical and physical properties of the platinum cured silicone gaskets and tubing have all been measured and benchmarked. All polymers used to construct the product contact components have met the requirements of a USP Class VI polymer and are regulated for food contact application according to 21 CFR Part 177 Indirect Food Additives. The specific polymer and regulatory information are summarized in Table 2.

Table 2. Polymer list and regulatory information

Component	Material	USP	21 CFR 177	Animal Origin	Additives
Column tubes OPUS 36R – 80R	70% w/w E-glass / PP homopolymer composite structure	Class VI USP <88>	177.1520	Animal-free	• Primary antioxidant • Processing aid
Column tubes OPUS 5 – 30	Extruded PP homopolymer	Class VI USP <88>	177.1520	Animal-free	• Primary antioxidant • Secondary antioxidant • Processing aid • Acid and metal scavengers
Flow distributors OPUS 2.5 – 80R	Compression molded PP homopolymer	Class VI USP <88>	177.1520	Animal-free	• Primary antioxidant • Secondary antioxidant • Processing aid • Acid and metal scavengers
Inlet and outlet ports OPUS R plug OPUS R inside port	Compression molded PP homopolymer	Class VI USP <88>	177.1520	Animal-free	• Primary antioxidant • Secondary antioxidant • Processing aid • Acid / metal scavengers
Bed support screens OPUS 2.5 – 80R	PP woven mesh	Class VI USP <88>	177.1520	EMA 410/01	• Primary antioxidant • Secondary antioxidant • Hindered amine • Processing aid • Acid and metal scavengers
Flow distributor O-rings OPUS R Plug O-ring Inlet/outlet gaskets	Platinum-cured silicone	Class VI USP <88>	177.2600	Animal-free	• Silica filler
Return line OPUS 10 – 80R	Platinum-cured silicone, PET braiding	Class VI USP <88>	177.2600	Animal-free	• Silica filler

Testing

The polymers utilized in medical and pharmaceutical applications should be compliant with the appropriate USP guidelines and it is recommended that they meet the USP Class VI testing requirements. The appropriate extractables and leachables (E&L) testing programs should be implemented for the bioprocessing materials that come into direct contact with components of the drug formulation. Three industry groups have published best practices for conducting this testing. Several presentations and publications were prepared by the Product Quality Research Institute (PQRI) for the evaluation and safety assessment of extractables and leachables in packaging for various drug dosage forms of high risk. These recommendations apply to the primary and secondary packaging associated with these pharmaceutical products. The BioProcess Systems Alliance (BPSA) and BioPhorum Operation Group (BPOG) have also published technical guideline for evaluating the risk associated with extractables and leachables specifically for single-use processing equipment.

The Repligen approach to E&L testing is that it is the responsibility of the product technology vendor to provide a technical extractable package. Leachables, on the other hand, are considered to be process specific and the responsibility of the end user to define with specific process solutions. With that in mind, Repligen has designed an extractables program to produce a robust data package by conducting extractables testing using five solvents under exaggerated conditions of contact. The data generated from this comprehensive series of extractables testing is detailed below.

The materials of contact in the OPUS Column platform:

- Extruded PP (2.5 – 30 cm tubes only)
- Polypropylene / E-glass composite (OPUS 36R – 80R tubes only)
- Machined compression molded PP (OPUS 2.5 – 80R Columns)
- Polypropylene mesh (OPUS 2.5 – 80R Columns)

- Platinum-cured silicone O-rings (OPUS 2.5 – 80R Columns)
- Platinum-cured silicone braided tubing (OPUS 10 – 80R Columns)

Repligen prepared samples of each component, and the third-party testing facility repurposed these samples into 2 g/20 mL for volatile analysis by headspace GC/MS and 2 g/10 mL for all other test methods.

Water and alcohols extractables test[1]

- Extraction solvents
  - USP water
  - 20% ethanol
  - 2.2% benzyl alcohol
- Extraction conditions
  - Elevated temperature: 40 +/- 3°C with continuous agitation for 72 hours.
- Analysis
  - The extracts were analyzed for semi-volatiles by GC/MS, non-volatiles by LC/PDA/MS, and inorganics by ICP/MS.
  - The neat OPUS components were analyzed for volatiles by headspace GC/MS.

Acid, base extractables test[2]

- Extraction solvents
  - M phosphoric acid (H3PO4)
  - 0.5 M sodium hydroxide (NaOH)
- Extraction conditions
  - Ambient: 25 +/- 3°C with continuous agitation at approximately 50 rpm for 72 hours.
  - Elevated temperature: 40 +/- 3°C with continuous agitation at approximately 50 rpm for 72 hours.
- Analysis
  - Extracts were analyzed for semi-volatiles by GC/MS, non-volatiles by LC/PDA/MS, and inorganics by ICP/MS.

3. Extractable Test Results for OPUS Columns

Results for the extracts are summarized in the tables below. Note the following:

- N/A indicates the compound was not observed at a reportable level in the sample extract.
- All other values are reported in µg/g.

3.1 Extruded PP: OPUS 2.5 – 30 cm Columns Only

Table 3. Volatiles by headspace GC/MS: extruded PP

Volatile Organic Compound (VOC)	Extractable
Hydrocarbon <C9	1.66 ug/g
Hydrocarbon <C9	1.47 ug/g

[1] Water and alcohols extractables tests were performed in 2014 prior to the final BPOG extractable guidance documents published in October 2015.

[2] The results for the acid-base extractables study were added after the original document drafted by Michael Ruberto, Ph.D. Acid and base extractables tests were performed in 2017 and utilized as many best practices as possible from the BPOG extractable guidance documents while attempting to maintain a level of consistency to the water and alcohols tests performed in 2014.

Source of extractables

The hydrocarbons can be traced back to low molecular weight oligomers present in the PP from incomplete polymerization or polymer degradation reactions. The percentages of low molecular weight oligomers have been benchmarked and are monitored for the OPUS Column polymer components to ensure a consistent extractables profile.

Table 4. Semi-volatiles by GC-MS: extruded PP

Extraction Conditions	Extractable (ug/g) None Detected
Water	N/A
2.2% benzyl alcohol	N/A
20% ethanol	N/A
0.1 M phosphoric acid, 25°C and 40°C	N/A
0.5 M sodium hydroxide 25°C and 40°C	N/A

Table 5. Semi-volatiles by GC/MS: extruded PP

Extraction Conditions	Extractable	
	Unknown	Unknown
Water	1.84 ug/g	2.86 ug/g
2.2% benzyl alcohol	1.83 ug/g	4.15 ug/g
20% ethanol	3.83 ug/g	10.19 ug/g
0.1 M phosphoric acid 25°C	N/A	N/A
0.1 M phosphoric acid 40°C	N/A	N/A
0.5 M sodium hydroxide 25°C	N/A	N/A
0.5 M sodium hydroxide 40°C	N/A	N/A

No non-volatile organic extractables were observed for the acid or base solvents.

Table 6. Inorganics by ICP/MS: extruded PP

Extraction Conditions	Extractable					
	Phosphorus	Sodium	Calcium	Silicon	Iron	Magnesium
Water	<4.51 ug/g	<1.13 ug/g	1.86 ug/g	4.02 ug/g	N/A	N/A
2.2% benzyl alcohol	<24.78 ug/g	1.92 ug/g	2.78 ug/g	1.42 ug/g	N/A	N/A
20% ethanol	<4.64 ug/g	<1.16 ug/g	2.78 ug/g	1.01 ug/g	N/A	N/A
0.1 M phosphoric acid 25°C	N/A	N/A	N/A	N/A	<1 ug/g	<1 ug/g
0.1 M phosphoric acid 40°C	N/A	N/A	<1 ug/g	N/A	1.32 ug/g	<1 ug/g
0.5 M sodium hydroxide 25°C	N/A	N/A	1.35 ug/g	N/A	N/A	<1 ug/g
0.5 M sodium hydroxide 40°C	N/A	N/A	5.27 ug/g	N/A	N/A	<1 ug/g

Source of extractables
Sodium and calcium can be traced back to the metal stearates and oxides used to scavenge residual catalysts and acid byproducts in the polymer. Silica is due to the siloxane-based lubricant used in the polymer manufacturing.

3.2 Machined Compression Molded PP: OPUS 2.5 – 80R

Table 7. Volatiles by headspace GC/MS: machine compression molded PP

Volatile Organic Compound (VOC)	Extractable
None observed	<1 ug/g

Table 8. Semi-volatiles by GC/MS: machine compression molded PP

Extraction Conditions	Extractable (ug/g) None Observed
Water	N/A
2.2% benzyl alcohol	N/A
20% ethanol	N/A
0.1 M phosphoric acid 25°C	N/A
0.1 M phosphoric acid 40°C	N/A
0.5 M sodium hydroxide 25°C	N/A
0.5 M sodium hydroxide 40°C	N/A

Table 9. Non-volatiles by UPLC/PDA/MS: machine compression molded PP

Extraction Conditions	Extractable			
	Unknown	Unknown	Unknown	Unknown
Water	2.74 ug/g	1.84 ug/g	2.75 ug/g	<1 ug/g
2.2% benzyl alcohol	<1 ug/g	<1 ug/g	4.14 ug/g	<1 ug/g
20% ethanol	1.93 ug/g	<1 ug/g	9.86 ug/g	4.38 ug/g
0.1 M phosphoric acid 25°C	N/A	N/A	N/A	N/A
0.1 M phosphoric acid 40°C	N/A	N/A	N/A	N/A
0.5 M sodium hydroxide 25°C	N/A	N/A	N/A	N/A
0.5 M sodium hydroxide 40°C	N/A	N/A	N/A	N/A

No non-volatile organic extractables were observed for the acid or base solvents.

Table 10. Inorganics by ICP/MS: machine compression molded PP

Extraction Conditions	Extractable					
	Phosphorus	Sodium	Calcium	Silicon	Iron	Magnesium
Water	<4.63 ug/g	<1.16 ug/g	2.23 ug/g	3.62 ug/g	N/A	N/A
2.2% benzyl alcohol	<4.77 ug/g	<1.20 ug/g	1.63 ug/g	1.69 ug/g	N/A	N/A
20% ethanol	<23.59 ug/g	1.41 ug/g	2.20 ug/g	<1 ug/g	N/A	N/A
0.1 M phosphoric acid 25°C	N/A	N/A	1.16 ug/g	N/A	<1 ug/g	<1 ug/g
0.1 M phosphoric acid 40°C	N/A	N/A	<1 ug/g	N/A	<1 ug/g	<1 ug/g
0.5 M sodium hydroxide 25°C	N/A	N/A	<1 ug/g	N/A	N/A	<1 ug/g
0.5 M sodium hydroxide 40°C	N/A	N/A	2.71 ug/g	N/A	N/A	N/A

Source of extractables

Sodium and calcium can be traced back to the metal stearates and oxides used to scavenge residual catalysts and acid byproducts in the polymer. Silica is due to the siloxane-based lubricant used in the polymer manufacturing.

3.3 PP Mesh: OPUS 2.5 – 80R

Table 11. Volatiles by headspace GC/MS: PP mesh

Volatile Organic Compound (VOC)	Extractable (ug/g)
None observed	N/A

Table 12. Semi-volatiles by GC/MS: PP mesh

Extraction Conditions	Extractable (ug/g) None Observed
Water	N/A
2.2% benzyl alcohol	N/A
20% ethanol	N/A
0.1 M phosphoric acid 25°C	N/A
0.1 M phosphoric acid 40°C	N/A
0.5 M sodium hydroxide 25°C	N/A
0.5 M sodium hydroxide 40°C	N/A

Table 13. Non-volatiles by UPLC/PDA/MS: PP mesh

Extraction Conditions	Extractable			
	Various Pegylated Compounds	Unknown	Unknown	Unknown
Water	1 – 150 ug/g	<1 ug/g	1.50 ug/g	<1 ug/g
2.2% benzyl alcohol	1 – 108 ug/g	1.12 ug/g	2.62 ug/g	<1 ug/g
20% ethanol	1 – 315 ug/g	2.06 ug/g	2.42 ug/g	3.84 ug/g

The pegylated extractables displayed a characteristic mass distribution of 44 amu increments. The empirical formulae for the prominent ions observed for these compounds ranged from $C_{15}H_{33}O_8$ to $C_{40}H_{82}NO_{13}$.

Table 14. Non-volatiles by UPLC/PDA/MS: PP mesh (continued)

Extraction Conditions	Extractable			
	PEG Compound with Formula $C_6H_{14}(C_2H_4O)_{6\text{-}11}$	Unknown	Unknown	Unknown
0.1 M phosphoric acid 25°C	N/A	N/A	1.01 ug/g	3.67 ug/g
0.1 M phosphoric acid 40°C	1.22 ug/g	N/A	1.36 ug/g	5.01 ug/g
0.5 M sodium hydroxide 25°C	N/A	N/A	N/A	N/A
0.5 M sodium hydroxide 40°C	N/A	1.34 ug/g	N/A	N/A

The unknown extractables in the acid-base study had potential formulas ranging from $C_{14}H_{28}O_2$ to $C_{14}H_{26}O_2$ and a mass distribution of 226 – 228 amu.

Table 15. Inorganics by ICP/MS: PP mesh

Extraction Conditions	Extractable					
	Phosphorus	Sodium	Calcium	Silicon	Iron	Magnesium
Water	<4.80 ug/g	2.74 ug/g	1.86 ug/g	3.23 ug/g	N/A	N/A
2.2% benzyl alcohol	<24.39 ug/g	5.09	2.32 ug/g	1.47 ug/g	N/A	N/A
20% ethanol	<4.95 ug/g	2.08 ug/g	1.83 ug/g	<1 ug/g	N/A	N/A
0.1 M phosphoric acid 25°C	N/A	N/A	<1 ug/g	N/A	<1 ug/g	<1 ug/g
0.1 M phosphoric acid 40°C	N/A	N/A	<1 ug/g	N/A	1.89 ug/g	<1 ug/g
0.5 M sodium hydroxide 25°C	N/A	N/A	4.47 ug/g	N/A	N/A	N/A
0.5 M sodium hydroxide 40°C	N/A	N/A	14.57 ug/g	N/A	N/A	<1 ug/g

Source of extractables

Sodium and calcium can be traced back to the metal stearates and oxides used to scavenge residual catalysts and acid byproducts in the polymer. Silica is due to the siloxane-based lubricant used in the polymer manufacturing.

3.4 P/E-Glass Composite: OPUS 36R – 80R Columns Only

Table 16. Volatiles by headspace GC/MS: P/E-glass composite

Volatile Organic Compound (VOC)	Extractable
Hydrocarbon <C9	2.84 ug/g

Source of extractables
The hydrocarbons can be traced back to low molecular weight oligomers present in the PP from incomplete polymerization or polymer degradation reactions. The percentages of low molecular weight oligomers have been benchmarked and are monitored for the OPUS Column polymer components to ensure a consistent extractables profile.

Table 17. Semi-volatiles by GC/MS: P/E-glass composite

Extraction Conditions	Extractable	
	None Observed	Diethyl Phthalate (CAS 84-666-2)
Water	N/A	N/A
2.2% benzyl alcohol	N/A	N/A
20% ethanol	N/A	N/A
0.1 M phosphoric acid 25°C	N/A	N/A
0.1 M phosphoric acid 40°C	N/A	1.48 ug/g
0.5 M sodium hydroxide 25°C	N/A	N/A
0.5 M sodium hydroxide 40°C	N/A	N/A

Diethyl phthalate (CAS 84-66-2) is commonly used as a plasticizer.

Table 18. Non-volatiles by UPLC/PDA/MS: P/E-glass composite

Extraction Conditions	Extractable					
	Pegylated Compound	Unknown	Unknown	Unknown	Unknown	Unknown
Water	<1 ug/g	24.15 ug/g	<1 ug/g	N/A	N/A	N/A
2.2% benzyl alcohol	<1 ug/g	<1 ug/g	<1 ug/g	N/A	N/A	N/A
20% ethanol	1.13 ug/g	<1 ug/g	1.01 ug/g	N/A	N/A	N/A
0.1 M phosphoric acid 25°C	N/A	N/A	N/A	N/A	N/A	N/A
0.1 M phosphoric acid 40°C	N/A	N/A	N/A	11.47 ug/g	4.52 ug/g	2.79 ug/g
0.5 M sodium hydroxide 25°C	N/A	N/A	N/A	N/A	N/A	N/A
0.5 M sodium hydroxide 40°C	N/A	N/A	N/A	N/A	N/A	N/A

The pegylated extractables displayed a characteristic mass distribution of 44 amu increments. Two mass distributions within the single chromatographic peak were detected. The empirical formulae for the prominent ions observed for these compounds ranged from $C_{15}H_{33}O_8$ to $C_{19}H_{44}NO_{10}$.

The unknown peak in the water extract was detected by LC/PDA. Ultra-performance liquid chromatography (UPLC) with fraction collection was used to isolate this unknown. The fraction was evaporated to dryness and re-analyzed by both GC/MS and LCMS;

however, no observable MS spectrum could be observed. NMR analysis of the fraction was inconclusive since significant signal could not be generated.

Table 19. Inorganics by ICP/MS: P/E-glass composite

Extraction Conditions	Extractable				
	Boron	Phosphorus	Sodium	Calcium	Silicon
Water	1.11 ug/g	<23.13 ug/g	6.62 ug/g	8.49 ug/g	25.99 ug/g
2.2% benzyl alcohol	1.94 ug/g	28.18 ug/g	5.82 ug/g	9.45 ug/g	30.92 ug/g
20% ethanol	1.32 ug/g	27.84 ug/g	9.45 ug/g	9.42 ug/g	21.18 ug/g

Table 20. Inorganics by ICP/MS: P/E-glass composite (continued)

Extraction Conditions	Extractable						
	Iron	Calcium	Magnesium	Aluminum	Phosphorus	Strontium	Silicon
0.1 M phosphoric acid 25°C	0.48 ug/g	2.67 ug/g	<1 ug/g	1.45 ug/g	N/A	N/A	N/A
0.1 M phosphoric acid 40°C	1.52 ug/g	6.24 ug/g	1.07 ug/g	5.03 ug/g	N/A	<1 ug/g	6.47 ug/g
0.5 M sodium hydroxide 25°C	N/A	19.93 ug/g	<1 ug/g	3.84 ug/g	13.76 ug/g	<1 ug/g	24.42 ug/g
0.5 M sodium hydroxide 40°C	N/A	43.05 ug/g	<1 ug/g	N/A	21.91 ug/g	1.58 ug/g	N/A

Source of extractables
Sodium and calcium can be traced back to the metal stearates and oxides used to scavenge residual catalysts and acid byproducts in the polymer. Silica, aluminum, boron, and phosphorus are common elements found in borosilicate and borophosphosilicate glass.

An analysis of strontium found in levels close to the Limit of Quantification (LOQ), confirmed the observed strontium was the ^{88}Sr and ^{86}Sr isotopes which are present in naturally occurring strontium containing minerals.

3.5 Platinum-cured Silicone O-ring: OPUS 2.5 – 80R Columns

Table 21. Volatiles by headspace GC/MS: platinum cured silicone O-ring

Volatile Organic Compound (VOC)	Extractable (ug/g)
None observed	N/A

Table 22. Semi-volatiles by GC/MS: platinum cured silicone O-ring

Extraction Conditions	Extractable (ug/g) None Observed
Water	N/A
2.2% benzyl alcohol	N/A
20% ethanol	N/A
0.1 M phosphoric acid 25°C	N/A
0.1 M phosphoric acid 40°C	N/A
0.5 M sodium hydroxide 25°C	N/A
0.5 M sodium hydroxide 40°C	N/A

Table 23. Non-volatiles by UPLC/PDA/MS: platinum cured silicone O-ring

Extraction Conditions	Extractable	
	Unknown	Unknown
Water	<1 ug/g	1.33 ug/g
2.2% benzyl alcohol	<1 ug/g	1.87 ug/g
20% ethanol	3.00 ug/g	4.32 ug/g

No non-volatile organic extractables were observed for the acid or base solvents

Table 24. Inorganics by ICP/MS: platinum cured silicone O-ring

Extraction Conditions	Extractable					
	Potassium	Phosphorus	Sodium	Calcium	Silicon	Iron
Water	<0.45 ug/g	<4.71 ug/g	<2.27 ug/g	1.18 ug/g	19.34 ug/g	N/A
2.2% benzyl alcohol	<0.44 ug/g	<22.48 ug/g	1.58 ug/g	1.37 ug/g	15.63 ug/g	N/A
20% ethanol	9.99 ug/g	<4.86 ug/g	<1.07 ug/g	1.08 ug/g	19.37 ug/g	N/A
0.1 M phosphoric acid 25°C	N/A	N/A	N/A	<1 ug/g	2.95 ug/g	1.38 ug/g
0.1 M phosphoric acid 40°C	N/A	N/A	N/A	1.32 ug/g	9.82 ug/g	1.71 ug/g
0.5 M sodium hydroxide 25°C	N/A	N/A	N/A	<1 ug/g	1,361.61 ug/g	N/A
0.5 M sodium hydroxide 40°C	N/A	N/A	N/A	<1 ug/g	1,478.06 ug/g	N/A

Source of extractables
A silica-based filler is used to provide enhanced mechanical properties to the platinum cured silicone polymer. The filler is the primary source of inorganic based extractables from this component.

3.6 Platinum-cured Silicone Braided Tubing: OPUS 10 – 80R Columns

Table 25. Volatiles by headspace GC/MS: platinum cured silicone braided tubing

Volatile Organic Compound (VOC)	Extractable
Decamethylcyclopentasiloxane	1.09 ug/g

Source of extractables
Decamethylcyclopentasiloxane is most likely present in the low molecular weight silicone-based processing lubricant used during tubing manufacturing.

Table 26. Semi-volatiles by GC/MS: platinum cured silicone braided tubing

Extraction Conditions	Extractable (ug/g) None Observed
Water	N/A
2.2% benzyl alcohol	N/A
20% ethanol	N/A
0.1 M phosphoric acid 25°C	N/A
0.1 M phosphoric acid 40°C	N/A
0.5 M sodium hydroxide 25°C	N/A
0.5 M sodium hydroxide 40°C	N/A

Table 27. Non-volatiles by UPLC/PDA/MS: platinum cured silicone braided tubing

Extraction Conditions	Extractable				
	Unknown	Unknown	Unknown	Unknown	Unknown
Water	30.73 ug/g	<1 ug/g	<1 ug/g	1.52 ug/g	N/A
2.2% benzyl alcohol	<1 ug/g	<1 ug/g	<1 ug/g	2.66 ug/g	N/A
20% ethanol	<1 ug/g	3.86 ug/g	1.91 ug/g	6.95 ug/g	N/A
0.1 M phosphoric acid 25°C	N/A	N/A	N/A	N/A	N/A
0.1 M phosphoric acid 40°C	N/A	N/A	N/A	N/A	N/A
0.5 M sodium hydroxide 25°C	N/A	N/A	N/A	N/A	2.02 ug/g
0.5 M sodium hydroxide 40°C	N/A	N/A	N/A	N/A	1.85 ug/g

The unknown peak in the water extract was detected by LC/PDA. UPLC with fraction collection was used to isolate this unknown. The fraction was evaporated to dryness and re-analyzed by both GC/MS and LC/MS; however, no observable MS spectrum could be observed. NMR analysis of the fraction was inconclusive since significant signal could not be generated.

Table 28. Inorganics by ICP/MS: platinum cured silicone braided tubing

Extraction Conditions	Extractable					
	Phosphorus	Sodium	Calcium	Silicon	Iron	Magnesium
Water	<4.71 ug/g	51.54 ug/g	1.27 ug/g	26.58 ug/g	N/A	N/A
2.2% benzyl alcohol	<22.48 ug/g	4.32 ug/g	1.32 ug/g	36.01 ug/g	N/A	N/A
20% ethanol	<4.86 ug/g	5.34 ug/g	1.56 ug/g	53.59 ug/g	N/A	N/A
0.1 M phosphoric acid 25°C	N/A	N/A	2.84 ug/g	3.62 ug/g	<1 ug/g	<1 ug/g
0.1 M phosphoric acid 40°C	N/A	N/A	<1 ug/g	10.37 ug/g	<1 ug/g	<1 ug/g
0.5 M sodium hydroxide 25°C	N/A	N/A	1.31 ug/g	126.84 ug/g	N/A	<1 ug/g
0.5 M sodium hydroxide 40°C	N/A	N/A	N/A	N/A	N/A	N/A

Source of extractables
A silica-based filler is used to provide enhanced mechanical properties to the platinum cured silicone polymer. The filler is the primary source of inorganic based extractables from this component.

3.7 Extractable Test Conclusions for OPUS Columns

The product contact materials used in OPUS Pre-packed Chromatography Columns meet the requirements of a USP Class VI plastic and are regulated for food contact applications. Each polymer has been hand selected based on its mechanical / physical properties, inherent stability, and low potential risk for leachables. Only those polymer additives that are functionally necessary are present in the OPUS product contact components. This has been confirmed experimentally through a complete quantitative analysis of the additives package in each polymer. Extractables testing has been conducted for the product contact components using conditions of 40°C for 72 hours in five solvents: USP water, 20% ethanol, 2.2% benzyl alcohol, 0.1 M phosphoric acid, and 0.5 M sodium hydroxide. The extracts were analyzed for volatiles, semi-volatiles, non-volatiles, and metals. The identified extractables were correlated back to their source of origin in the OPUS component so that they can be controlled during manufacturing. Structure elucidation was performed on the unknowns and empirical formulae were proposed when possible. The physical properties of the polymers that have the most influence over leachables (such as melting point, glass transition temperature, percent crystallinity, and molecular weight distribution) have been benchmarked to ensure a consistent batch-to-batch leachables profile for these components and a well-managed supply chain for raw materials used in their construction. The result is a chromatography column which has achieved a minimization and control of leachables through comprehensive material characterization, quality control, and extractables testing.

3.8 References

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4. Index

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